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CASE REPORT

No effect of immunomodulatory therapy in focal epilepsy with positive glutamate receptor type 3—antibodies

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Summary Antibodies against the glutamate receptor type 3—(GluR3) have been found in association with Rasmussen's encephalitis (RE) but were also detected in patients with non-inflammatory focal epilepsies. We report the case of an 18-year-old patient with treatment refractory left mesial temporal lobe epilepsy accompanied by high levels of GluR3 antibodies. Different from experiences in patients with RE immunomodulatory therapy by use of intravenous gammaglobulines neither altered GluR3 serum levels nor had any effect on seizure frequency in our patient. Interestingly, GluR3 serum levels remained positive after successful surgical intervention leading to patient's seizure freedom.

Introduction

The pathogenic role of humoral or cellular autoimmune mechanisms for the development of epileptic seizures is not well understood.[1] Strongest evidence for a direct involvement of the immune system in epileptogenesis has been found in Rasmussen's encephalitis (RE), a rare condition presenting with intractable partial seizures, unilateral progressive brain dysfunction and inflammatory histopathological changes which may be accompanied by antibodies against the ionotrophic glutamate receptor (GluR3).[2] Therapeutic concepts of this rare syndrome include — besides early resective surgery — plasma exchange, immunosuppressive drugs, and immunomodulation using intravenous administration of high-dose gammaglobulines (IVIG).[3,4]

Recently, it was reported that GluR3 antibodies were non-specific for RE as they were also found in some sera of patients with focal epilepsies.[5,6] It has, therefore, been speculated that — similar to

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RE — these antibodies might play a role in provoking seizures in severe focal epilepsies, too. Different pathogenetic mechanisms of these antibodies are discussed: on the one hand, they may have an excitotoxic glutamate-like potential as they were shown to induce glutamate receptor like ion currents.[7] On the other hand, GluR3 antibodies were described to possess a neuronal-killing potential via complement-dependent cytotoxicity mechanisms.[8]

As IVIG therapy is reported to be an effective therapeutic regimen for patients with RE it may be speculated that it has then also positive effects on seizure frequency in patients with severe focal epilepsy that are associated with positive GluR3 antibodies. Recently, in one child a short-lasting IVIG therapy was ineffective.[9] In this report we present another patient (with focal temporal lobe epilepsy and highly positive GluR3 antibodies) who was treated with IVIG over 3 months.

Case report

The patient is an 18-year-old left-handed man whose delivery was prolonged and complicated (rupture of the uterus, Apgar score 3/6/9). His subsequent psychomotor development was normal and no neurological deficits were apparent. The family history was negative for epilepsy and other neurological disorders. At age 15, several weeks after an antibiotic treatment of a sinusitis, he developed short-lasting complex partial seizures (CPS) with an initial perception of fear, oral automatisms, speech arrest, a left-sided piloerection and a loss of consciousness. Over the next few months, the frequency of these focal seizures increased—in some periods up to 30—50 CPS/day. Response to various combinations of high-dose carbamazepine, valproic acid, phenytoin, clonazepam and levetiracetam was poor.

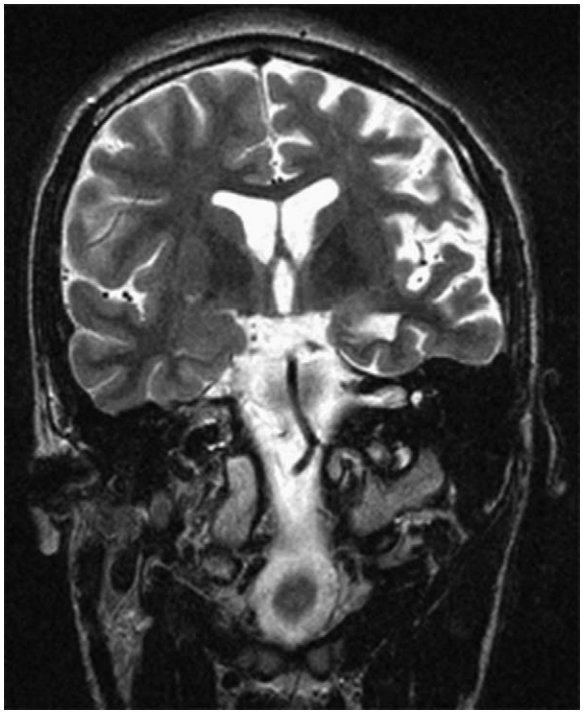

Figure 1 Coronal T2-weighted MRT image of the patient with gyral atrophy of the left frontal, parietal and temporal cortex as well as left hippocampal volume loss.

Repeated electroencephalograms revealed frequent interictal spikes over the left temporal region. Ictal EEG during video-EEG monitoring showed focal rhythmic spike-wave discharges in the left temporal and frontal electrodes (F7, F3, T5, T7, and TP9). MRI showed a diffuse atrophy of the entire left hemisphere including the hippocampal formation (see Fig. 1) with no apparent progression over 6 months. An ictal ^{99}Tc-m HMPAO-SPECT scan demonstrated a focally restricted increase of tracer uptake in the left temporo-mesial region (see Fig. 2). Neuropsychological testing revealed significant deficits in the verbal memory compared to the

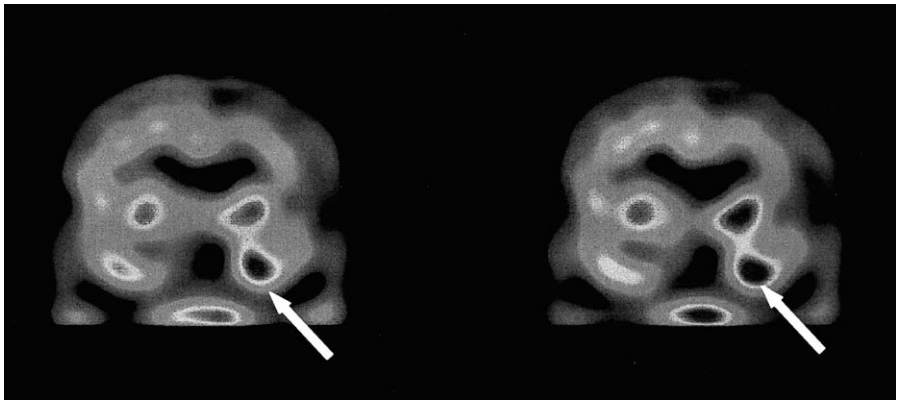

Figure 2 Ictal ^{99}Tc-m HMPAO scan (coronal slice) with focal tracer enhancement in the left mesio-temporal region and the left basal ganglia (white arrow).

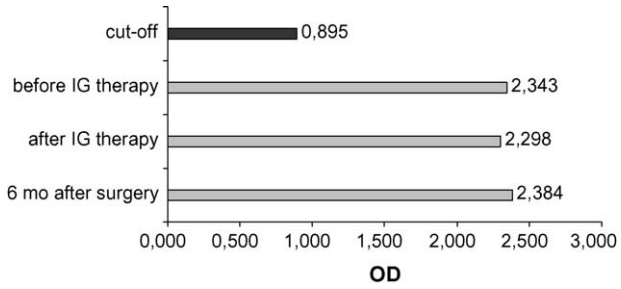

Figure 3 Time course of serum reactivity against GluR3B1-peptide. ELISA optical densities (OD) with patient sera at dilution of 1:10 are reported. Titers of the patient (i) before; (ii) after three monthly cycles of intravenous therapy with gammaglobulines (IVIG); and (iii) 6 months after successful surgery (seizure freedom) are represented as OD values (grey bars) for GluR3B1 reactivity. Note that titers remain elevated after IVIG therapy and successful surgery. Black bar (left) shows in comparison the cut-off value for positivity (mean normal controls $\pm$ 3 S.D., black bar).

visual memory and a bilateral language representation (WADA test).

Due to the clinical symptomatology with a high seizure frequency up to occasional series of CPS over days and the left hemispheric atrophy, RE was considered as differential diagnosis of focal temporal epilepsy. CSF contained 1 lymphocyte/mm^3, had no oligoclonal bands, and total protein and glucose were normal. The patient's serum was investigated for antibodies against glutamate receptors type 3 (GluR3) which were highly positive (for details see below).

Although a defined epileptic focus within the left mesio-temporal region could be found, the patient was sceptical concerning a surgical procedure. A high-dose IVIG therapy was started which consisted of three monthly cycles (0.4 g/kg/day each month over 5 days). Seizure frequency was completely unaffected by this regimen. The patient, therefore, consented to undergo left-sided partial lobectomy (including amygdalo-hippocampectomy) and histological analysis for reliable exclusion of RE was available. The histopathological specimen showed no inflammatory process indicative of RE but signs of a cortical dysplasia with heterotopic cells in the white matter. Postoperatively, the patient was seizure free with a follow-up period of 2 years now (Engel class I A). Both, after the end of IVIG therapy (three monthly cycles) as well as 6 months after surgery, the patient's serum was controlled for levels of GluR3 antibodies. GluR3 antibody titers including the epitope recognition pattern of the complete GluR3 sequence[6] remained unchanged over time (see Fig. 3).

GluR3 peptides and anti-GluR3-antibody-ELISA

Different immunodominant regions of the GluR3-receptor (GluR3A1, GluR3A2, GluR3B1, GluR3B2, N-terminal, hydrophilic domain, cytoplasmic domain, and C-terminal) were chosen for testing antibody reactivities.[6] ELISA for the detection of peptide-specific GluR3 antibodies was performed as described previously.[6] Fig. 3 shows the time course of antibody reactivities against GluR3B1. Optical density (OD) values were 2.343 before and 2.290 after IVIG therapy, and 2.384 six months after surgery. The cut-off for anti-GluR3B1 positivity (0.895 OD) was defined as mean OD $\pm$ 3 S.D. of the controls (''CNS-normal'' and healthy donors: 0.419 $\pm$ 0.476) as described recently.[6] Of note, the patient exhibited strong reactivities also against other peptides of the GluR3-receptor including the cytoplasmic domain. Neither absolute values nor the recognition pattern of the antibodies changed over time (data not shown).

Discussion

Concerning the clinical course and the therapy of our patient three observations are remarkable. First, the presence of GluR3 in the serum without histopathological proof of RE corroborates recent work reporting that these antibodies are non-specific for RE.[5,6] Although the clinical picture of the epileptic seizures did not resemble epilepsia partialis continua and neurological deficits were not prominent in our patient, the frequent series of CPS of temporal origin were reminiscent of RE—the more so as there is some evidence in the literature for a possible onset of the inflammatory process of RE in the temporal lobe.[10] The most likely underlying cause of the development of seizures in our patient, however, is a cortical migration disorder (based on the histopathological analysis). According to the patient's history and the cranial MRT an additional diffuse left hemispheric perinatal hypoxic damage may be responsible for the seizures too, but this

remains speculative. RE could be excluded as no inflammatory signs were detectable histopathologically and repeated MRI scans showed no progressive disorder. The high seizure frequency in RE and in focal epilepsies accompanied by positive GluR3 antibodies is commonly viewed as a result of an initial blood-brain barrier disruption—caused by different possible mechanisms (e.g. trauma, seizure).[11] This allows circulating antibodies to enter into the brain producing further neuronal injuries, subsequent seizures and a constant focal blood-brain barrier disruption. This ''vicious circle'' leading to recurrent seizures may be entertained by GluR3 antibodies—either via direct agonistic receptor activation[7,12] or by complement-dependent cytotoxicity mechanisms.[8] Reports of clinical improvement in response to elimination of GluR3 antibodies after plasmapheresis support the assumption of these autoantibodies being causally involved in pathogenetic process of RE.[13] Whether a tendency to intractable series of seizures in cryptogenic focal epilepsies with elevated serum GluR3 levels could be provoked by similar (auto) immune mechanisms remains speculative.

Secondly, in patients with RE seizures are reported to decrease after therapy with IVIG[4] but in our patient suffering from a cryptogenic focal epilepsy this immunomodulatory treatment showed no effect—neither on seizure frequency nor on GluR3 serum titers. This is consistent with one recent case report[9] of a child with focal epilepsy and positive GluR3 autoantibodies. One reason for the therapeutic failure of IVIG in both patients may be that one single treatment cycle in the child's case[9] and our 3 months treatment, respectively, has been too short as in some patients with RE improvements after immunomodulation were also delayed.[4] Due to multifaceted mechanisms, IVIG modulate mainly humoral but also cellular immune responses.[14] Possible mechanisms of action that modulate humoral immune response may be (i) neutralization of or competition with the binding site of anti-GluR3 antibodies by anti-idiotype antibodies; (ii) binding to complement factors or reduction of activated complement deposition with inhibition of complement-mediated neuronal damage; and (iii) down regulation of the production of GluR3 due to antibodies against B cells. In our patient, GluR3 antibody levels after IVIG therapy remained unchanged arguing against the hypothesis of a suppressed GluR3-production by B-cells following this treatment strategy.

Our third observation of remaining high levels of GluR3 antibodies 6 months after successful surgery — despite of total cessation of seizures and without interictal epileptic discharges on scalp EEG — was surprising. If GluR3 antibodies were causally involved in the epileptogenesis and their synthesis thus be triggered by recurrent seizures and neuronal damage, one would expect a decrease of antibody titers in the absence of the triggering focus. Remaining high levels of GluR3 antibodies may therefore be rather interpreted as an unspecific (epi-) phenomenon in a subset of non-inflammatory focal epilepsy patients with rather stable levels—independent from seizure frequency.

The current case provides insight on the effect of immunomodulatory IVIG therapy in GluR3 positive patients with non-inflammatory focal epilepsy: (i) immunomodulatory IVIG therapy was not beneficial in our patient but may have been if it were offered longer than 3 months. (ii) GluR3 antibody titers were not correlated to seizure frequency and remained consistently high despite removal of the epileptic focus. In this case GluR3 antibodies are highly unlikely to represent a causative and therefore treatable factor of epileptogenesis. However, further studies are needed to clarify the pathogenetic relevance of GluR3 in patients with focal epilepsies and the use of immune oriented treatment strategies in this subgroup of patients.

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